DATA EVALUATION RECORD
HONEY BEE - FIELD TESTING FOR POLLINATORS

i 141-5 (OPPTS 850. 3040)

1. CHEMICAL: Clothianidin PC Code No.: 044309

2. TEST MATERIAL: Clothianidin FS 600B G Purity: 595 g/L

3. CITATION:

Author: Liepold, K.

Title: Monitoring of potential effects of the drilling of clothianidin treated maize seeds on honeybees, guttation monitoring of maize seedlings under agronomic use conditions and assessment of the relevance of guttation for honeybees in Languedoc-Roussillon (France).

Study Completion Date: January 6, 2010

Laboratory: Eurofins-GAB GmbH, Niefern, Oschelbronn, Germany

Sponsor: Bayer CropScience AG, Ecotoxicology, Monheim, Germany

Laboratory Report ID: S09-01404

DP Barcode: 374484

MRID No.: 47972301

4. REVIEWED BY: Moncie Wright, Staff Scientist, Cambridge Environmental, Inc.

Signature: Moncie V Wright **Date:** 08/02/11

APPROVED BY: Teri S. Myers, Ph.D., Senior Scientist, Cambridge Environmental Inc.

Signature: Teri S Myers **Date:** 08/02/11

5. APPROVED BY: Allen Vaughan, Biologist, ERB - V

Signature: **Date:**

6. DISCLAIMER: This document provides guidance for EPA and PMRA reviewers on how to complete a data evaluation record after reviewing a scientific study concerning the long-term toxicity of a pesticide to honey bees following an actual-use field exposure. It is not intended to prescribe conditions to any external party for conducting this study nor to establish absolute criteria regarding the assessment of whether the study is scientifically sound and whether the study satisfies any applicable data requirements. Reviewers are expected to review and to determine for each study, on a case-by-case basis, whether it is scientifically sound and provides sufficient information to satisfy applicable data requirements. Studies that fail to meet any of the conditions may be accepted, if appropriate; similarly, studies that meet all of the conditions may be rejected, if appropriate. In sum, the reviewer is to take into account the totality of factors related to the test methodology and results in determining the acceptability of the study.

7. STUDY PARAMETERS:

Scientific Name of Test Organism:	*Apis mellifera* L.
Age or Size of Test Organism at Test Initiation:	Queens in all colonies were of the same lineage and the bees in all colonies were young.
Definitive Study Duration:	81 days (4-day pre-exposure period and 45-day exposure period followed by a 32-day post-exposure period).

8. CONCLUSIONS:

In an 81 day study (4-day pre-exposure period and 45-day exposure period followed by a 32-day post-exposure period), the toxicity of dust from clothianidin-treated seed during drilling of treated maize seeds was examined in the honey bee, *Apis mellifera* L., under open field conditions at two test sites (the treatment plots were located near Nimes), in the region of Languedoc-Roussillon, France. The treated site was planted with maize seeds dressed with the end-use product Clothianidin FS 600B G (AI: 595 g/L Clothianidin or 0.500 mg a.s./seed), and the other site was planted with untreated control seed. The treatment and the control plots were separated by *ca*. 3.3 km. The maize seeds were sown at a nominal drilling rate of 2 units (100,000 seeds)/ha. Six honeybee colonies were placed at the edge of the each field plot at a distance of 1-2 m from the sowing area with the entrance facing the maize field.

The colonies were established in a downwind position relative to the field in order to maximize potential dust exposure during drilling. The colonies were placed in the fields 4 days before the drilling of the maize seeds and remained at the study location for 45 days after seeding.

For the post exposure period the colonies were moved from the exposure plots to a monitoring location near Bellegarde, Languedoc-Roussillon, France. Throughout the study, colonies were assessed for mortality, colony strength, and brood and food store area. Additionally, the occurrence and duration of guttation, flight activity and bee behavior, and bees collecting guttation liquid were also observed.

The proportion of guttating plants varied from 0 to >90% of all plants in the assessed areas of both the control and treatment plots. In general, guttation occurred at a similar rate over the 4 zones that were assessed, but not at a similar rate between the control and treatment group. In general, the occurrence of guttation was slightly more frequent in the treatment plot. Days where strong guttation occurred were observed in June in both the control and treatment plots. Dew and guttation did not occur together on all assessment days. Generally, there were more days with guttation only than with both guttation and dew.

No honeybees were observed consuming guttation liquid or otherwise interacting with guttation liquid droplets in the control or treatment plot for the entire duration of the study period. Flight activity early in the morning was slightly lower in the control plot compared to the treatment plot. Flight activity increased during the course of the morning in both plots, and flight activity in the control and treatment plots was then comparable. The period of guttation and bee activity overlapped. Bee behavior in the front of the hives was normal in the both the treated and control plots.

The daily mean pre-exposure (days -3 to -1) mortality (linen sheets + dead bee traps) in the control and treatment groups was 32.8 and 39.9 bees/hive, respectively. On the day of drilling (but after the process was complete), mortalities averaged 40.5 bees/hive in the control field as compared to 36.5 bees/hive in the treated field. For the remaining assessment days, mean daily mortality of both the control and treatment groups demonstrated the same tendency to fluctuate and also demonstrated comparable timings of increases and decreases. Mortality peaks usually occurred simultaneously in both the control and treated plots and were usually higher in the control plots. The mean daily mortality during guttation (May 28-June 26, 2009; days 6-35 after drilling) was 42.6 and 38.4 dead bees/hive in the control and treated groups, respectively. The mean daily mortality (linen sheets + bee traps) for the entire exposure (45 days) was 39.6 and 35.7 bees/hive in the control and treated groups, respectively.

At the first brood assessment, colony strength (=mean number of bees/hive) in the control hives ranged from 7,329 to 12,236 bees. Colony strength in the treatment hives ranged from 5,985 to

12,502 bees. Only the bees that were present in the hives at the time of the assessment were included in the estimates. Colony strength in both the control and treatment group were similar during the first brood assessment. The colony strength in the treatment group was comparable to that of the control group during pre-exposure and for many exposure assessments. There were 4 assessments where the control group had greater colony strengths as compared to the treatment group that might have been biologically significant. However, the time of assessment likely affected the available number of bees for counting at the hives as the treatment group was measured during a time of higher bee flight activity as compared to the control. Therefore, any reductions cannot be conclusively attributed to the drilling of the treated maize seeds.

The development of the mean abundance of brood on the combs (eggs, larvae, and pupae) in the control was slightly higher from June 11 until the last assessment beginning in August (Figures 3 and 4). However, brood development followed the same trends in both the control and treatment group, and the values were reportedly within the range of natural variation.

The comparatively lower abundance of brood in the treatment hives in June and the beginning of July was reportedly caused by hives T2, T4, and T5. In hive T2, no queen was observed as present during two brood assessments (July 2 during exposure and July 9 after relocation), which could explain the low brood abundance.

There were likely no biologically significant reductions in the treatment group present during pre-exposure with the exception of the sum area of egg cells; however, there was no statistically significant reduction for this endpoint. The treatment group had noticeably higher sum areas of pollen and pupal cells as compared to the control during pre-exposure. Statistically significant reductions were determined for the sum area of egg cells on 34 and 63 days after drilling; however, the treatment group was already reduced in comparison to the control group before the exposure was initiated. The reviewer could not definitively determine whether the reductions could be attributed to clothianidin-dressed maize seeds.

There was high variability present in this study that precluded the ability of the t-tests to indicate statistical significance. As a result, there are limitations on the both the results and the reviewer's ability to determine if there was in fact a treatment related effect of clothianin-dressed maize seed on honeybees.

The reviewer concludes that the data presented in this study are inadequate to accurately determine the effects of clothianidin-treated maize seedlings on honeybees and colony health. Guttation fluid, dead honeybees and pollen and nectar from combs were not analyzed because the study authors determined there was no damage to individual bees or bee colonies due to clthianidin-treated maize exposure.

This study is scientifically sound and *satisfies/does not satisfy* the EFED concerning the guideline requirements for a field toxicity test with honeybees (Subdivision L, i 141-5 or 850.3040).

9. ADEQUACY OF THE STUDY:

A. Classification: Acceptable / Supplemental / Unacceptable

B. Rationale: N/A

C. Repairability: N/A

10. GUIDELINE DEVIATIONS: There were no guideline deviations.

11. SUBMISSION PURPOSE: This study was submitted to provide data on the toxicity of clothianidin to honeybees in a field test for the purpose of chemical reregistration.

Specifically, the test was conducted to determine the relevance of potentially occurring guttation in young maize plants in the Languedoc-Roussillon region in France as a water source for honeybees, and to assess potential effects of Clothianidin residues from the seed treatment of the maize seeds in guttation liquid on bee colonies under field conditions. Additionally, assessments were performed on the potential effects of the maize drilling process during which the colonies might be exposed to Clothianidin-containing dust from the seed treatment.

12. MATERIALS AND METHODS:

A. Test Organisms

Guideline Criteria	Reported Information
Species: **Species of concern (*Apis mellifera*, *Megachile rotundata*, or *Nomia melanderi*)**	*Apis mellifera* L. (Hymenoptera, Apidae)
Colony description at beginning of test:	Each colony occupied hives with 1 box consisting of 10 combs each.

Guideline Criteria	Reported Information
	The colonies were of the same lineage and approximately the same age. There was 1 queen per colony and between 5,985 and 12,502 bees per colony at study initiation.
Pre-test health:	Bees were reportedly free of *Nosema* and *Varroa* disease symptoms.
Supplier	The colonies were supplied by Syntech Research, France.
All bees from the same source?	Yes

B. Test System

Guideline Criteria	Reported Information
Exposure Site Location and Establishment:	The test fields were located near Nimes, in the region of Languedoc-Roussillon, France. The treated site was planted with clothianidin-dressed maize seed and the other planted with untreated control seed. The treatment and the control plots were separated by *ca*. 3.3 km. The size of the field plots was 2.1 ha for the treated plot and 2.8 ha for the control. The maize seeds were sown at a nominal drilling rate of 2 units (100,000 seeds)/ha. Effective rates: Control: 104,400 seeds/ha Treatment: 108,200 seeds/ha
Site Preparation:	None reported.
Number of applications:	One; drilling occurred on May 22, 2009 after the hive set-up on the plots 4 days prior.
Number of Replicates/Treatment:	Six colonies per field plot, with 1 treated and 1

Guideline Criteria	Reported Information
	control field plot
Post-exposure Site Location:	Near Bellegarde, Languedoc-Roussillon, France.
Lighting:	Natural; not further described.
Precipitation:	Precipitation measured during mortality assessments at the control plot ranged from *ca*. 0.0 to 52 L/m^2 and 0 to 46 L/m^2 in the treatment plot during the exposure period (May 19 to July 3, 2009; data obtained from Figure 23). The maximum rainfall event occurred around June 8, 2009 when 45 (treatment) and 52 (control) L/m^2 precipitation occurred.
Temperature:	Daily temperatures ranged from 10.8 to 36.3°C after hive set-up and during the entire exposure period (May 18 to July 6, 2009).
Relative humidity:	Mean relative humidity ranged from 32 to 81% after hive set-up and during the entire exposure period (May 18 to July 6, 2009).

C. Test Design

Guideline Criteria	Reported Information
Range finding test?	None reported
Reference toxicant tested?	No
Duration of Exposure Period	45 days
Duration of Post-exposure Period	32 days in the monitoring site
Test Substance(s):	Clothianidin FS 600B G Formulation Type: suspension Batch No.: PF90191228 AI: 595 g/L Clothianidin (analyzed)
Control Substance(s):	N/A- control seeds were not treated

Guideline Criteria	Reported Information
Maize Seed:	Seed variety: DKC5166
Application Rate:	0.506 mg ai per seed (analyzed)
Verification of Application Rate:	Method not reported
Method of Seed Coating:	Not reported
Colony Introduction:	The colonies were placed at the edge of each field plot at a distance of 1-2 m from the sowing area with the entrance facing the maize field. The colonies were established in a downwind position relative to the field in order to maximize potential dust exposure during drilling. The colonies were placed in the fields 4 days before drilling and remained at the study location for 40 days after seedling emergence.

Guideline Criteria	Reported Information
Post-exposure:	The colonies were moved from the exposure plots to a monitoring location near Bellegarde, Languedoc-Roussillon, France.
Assessment scheme:	The part of the field plots that was considered to be most likely to be attractive to honeybees seeking water was assessed regarding the occurrence of guttation and/or dew (assessment area). The in-field assessment area (zones 1-4) covered a width of 5 m to the left and to the right from the outer bee hives at each field, and in length encompassed 58 parallel rows of maize (41.6 m). Each assessment started with zone 0 and ended with zone 4.
Assessment zones:	Zone 0 = off-field assessment area; between row number 1; 2-4 m away from the field. Zone 1 = rows 1-7; assessments were performed along each row; observers made assessments while walking. Zone 2 = rows 8-13; assessments were made for rows in groups of 3 (each 3rd row was a passing row). Zone 3 = rows 14-28; assessments were made for rows in groups of 5 (each 5th row was a passing row). Zone 4 = rows 29-58; assessments were made for rows in groups of 5 (each 10th row was a passing row). Additionally, there were six 2 m^2 plots that each covered 2 rows of maize seedlings.

D. Biological Assessments

Guideline Criteria	Reported Information
Maize guttation:	The proportion of maize plants displaying guttation and/or dew was monitored for a

Guideline Criteria	Reported Information
	period of 40 days from the emergence of maize plants, until July 6, 2009, when no further guttation could be observed over 10 days and the hives were removed from the exposure site. Guttation started 2 days after emergence of the maize plants. Guttation was assessed by observers that walked through each passage row. The percentage was estimated at 10, 25, 50, 75, 90, and >90%. If less than 10% of the plants displayed guttation, the exact number of plants in an assessment row that showed guttation was counted. Guttation occurrence was checked in regular intervals from the early morning onwards until no more guttation drops were visible. One full observation period included the guttation assessments in the 4 established zones in the fields. Additionally, zone 0 was checked for the presence of guttation and/or dew on the off-field vegetation and to determine if the extent of guttation and/or dew on the off-field vegetation was more or less than that present on the plants in the maize field. If no guttation occurred at both field sites then the plants of neighboring fields or adjacent vegetation (in addition to zone 0) were checked for guttation.
Bees collecting guttation droplets:	After the assessment of guttation and honeybee activity in the zones the number of honeybees per assessment plot (2 m^2) sitting on the ground or on plants, and the number taking up droplets was recorded during a 4 minute assessment period per plot. Any abnormal behavior was documented. Additionally,

Guideline Criteria	Reported Information
	observations were conducted in the walking rows of zones 0-4.
Flight activity:	On each assessment day (those days on which guttation was observed), the flight activity at the hive entrance of each hive was documented at the start and end of each observation period. Flight activity was assessed by counting the number of bees entering the hive over 1 minute and by counting the number leaving the hive over 1 minute.
Mortality:	Linen sheets were spread on the ground in front of the hives and dead bee traps were attached to the entrance of each hive to measure mortality during the exposure period. Mortality was assessed four days before drilling, on the day of seeding (after seeding was done), and daily thereafter until the termination of the exposure phase. The dead bee traps were emptied daily at the same time of day and the bees were transferred within 10 hours into a deep freezer (≤-18°C) for potential residues analysis.

Guideline Criteria	Reported Information
Colony condition:	The condition of the colonies was recorded once before the hives were placed on the field plots and afterwards in weekly intervals during the exposure phase.
Brood:	During the monitoring phase the brood assessments were performed 5 times in weekly intervals. The following parameters were assessed: - Colony strength (number of bees) - Presence of a healthy queen (presence of eggs) - Pollen storage area and area with nectar or honey - Area containing cells with eggs, larval, and capped cells The comb area covered with bees and containing cells with nectar, pollen, egg, larval, and capped cells was estimated per comb side and the total number of bees and cells containing the brood stages, pollen, and nectar on the comb was calculated. The mean values were calculated for each hive and assessment date.
Collection of guttation fluid:	Guttation fluid was sampled on days when sufficient guttation for sampling was available early in the morning in the treated plot. The samples were collected in the morning within the first hour of the assessments on the field outside the guttation assessment areas and in a distance of at least 20 m from the hives. The fluid was collected with plastic Pasteur pipettes and was stored in Eppendorf caps. Samples were stored on blue ice and transferred within 14 hours to a deep freezer (≤-18°C). During the trial, sampling occurred

Guideline Criteria	Reported Information
	on 21 days.

Guideline Criteria	Reported Information
Collection of pollen and nectar from combs:	Samples of pollen and nectar were collected from the bee hive combs during each brood assessment after drilling during the exposure phase. If possible, one sample that weighed 1 gram was taken per colony in the control and treated plots. Each sample was taken from 3 different sections per hive, and then all 3 samples were pooled. Pieces of comb were cut from the comb using a clean knife for each sample. A spoon was used to collect nectar. Samples were stored cooled and transferred within 10 hours to a deep freezer (≤-18°C). No further preparation was performed because the residues were not analyzed.

E. Residue Analysis

Guideline Criteria	Reported Information
Guttation fluid, dead bees, pollen and nectar from combs:	The study author concluded that Clothianidin-treated maize did not have negative effects on any of the biological endpoints measured; therefore, the author deemed it unnecessary to perform residue analysis.

13. REPORTED RESULTS:

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Signed and dated No Data Confidentiality, GLP, and Quality Assurance Statements were provided. This study was conducted in compliance with the most recent edition of the Principles of Good Laboratory Practice, Chemikaliengesetz, Attachment 1, Germany, and the OECD Principles of Good Laboratory Practice.

Guideline Criteria	Reported Information
	The German requirements are based on the OECD Principles of GLP, which are accepted by regulatory authorities throughout the European Community, the United States of America (FDA and EPA) and Japan (MHW, MAFF, and METI) on the basis of intergovernmental agreements. This study was not conducted according to any established guidelines; therefore, it was performed according to the study plan and SOPs of eurofins-GAB.
Raw data included?	Yes
Signs of toxicity (if any) were described?	Yes

Observations of guttation and proportion of guttating plants:
Guttation was observed for a total of 21 and 24 days in the control and treatment plot, respectively. However, assessments were only carried out on 20 days in the control plot and on 22 days in the treatment plot as there were some days where guttation only occurred in the evening after bee flight. Guttation assessments totaled 32 and 37 in the control and treatment plots, respectively.
In the morning, guttation generally ended between 7:45 and 11:15 am. Guttation was observed in the evening on 12 and 15 days in the control and treated plots, respectively. Guttation on adjacent vegetation and on neighboring fields was observed on most days when guttation occurred on the maize plots in the control and treatment groups.
The proportion of guttating plants varied from 0 to >90% of all plants in the assessed areas of both the control and treatment plots. In general, guttation occurred at a similar rate over the 4 zones that were assessed, but not at a similar rate between the control and treatment group. In the control, there were 18 days with a maximum of >50% of the plants displaying guttation, and 2 days with a maximum of 50% of the plants displaying guttation. In general, the occurrence of guttation was slightly more pronounced on the treatment plot. There were 21 days with a maximum of >50% of the plants displaying guttation and 1 day with a maximum of 50% of the plants displaying guttation. Days where strong guttation occurred were observed in June in both the control and treatment plots. Dew and guttation did not occur together on all assessment days. Generally, there were more days with guttation only than with both guttation and dew. Guttation in zone 0 was less pronounced than on the maize crop in the control. In the treatment plot, the frequency and extent of guttation in zone 0 varied less and more compared to the maize crop.

However, there were more days with less pronounced occurrences of guttation or dew in zone 0 than in the maize crop.

Honeybees visiting plants displaying guttation:
During the assessment of guttation in the control plot, 1-2 single bees per assessment were observed sitting on maize plants in 6 out of 35 assessments, and 1 and 4 bees per assessment were found sitting on the ground during 2 out of 35 assessments. In the 2 m^2 observation areas, bees were found sitting on the ground or on plants in 3 out of 33 assessments (1 single bee per area).

In the treated plot, between 1 and 16 bees per assessment were observed sitting on plants or on the ground for 3 of 37 assessments. In the 2 m^2 areas, bees were on the ground or on plants for 7 out of 33 assessments (1-2 bees per area).

No honeybees were observed consuming guttation liquid or otherwise interacting with guttation liquid droplets in the control or treatment plot for the entire duration of the study period.

Flight activity:
Flight activity early in the morning was slightly lower in the control plot compared to the treatment plot. Flight activity increased during the course of the morning in both plots, and flight activity in the control and treatment plots was then comparable. The period of guttation and bee activity overlapped. Bee behavior in the front of the hives was normal in the both the treated and control plots. No behavioral anomalies were observed.

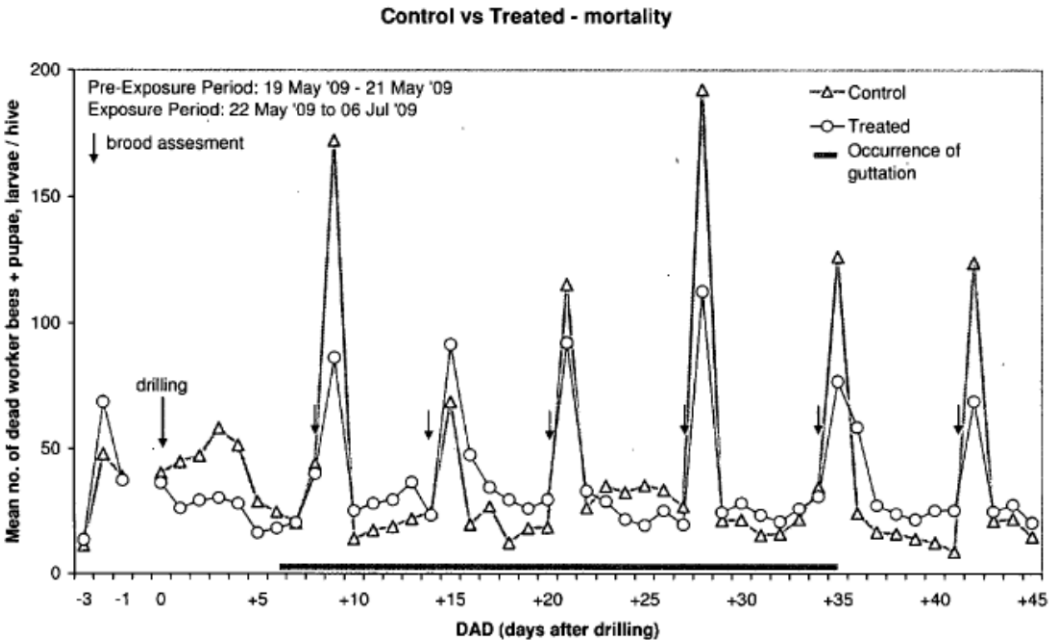
Mortality:
The daily mean pre-exposure (days -3 to -1) mortality (linen sheets + dead bee traps) in the control and treatment groups was 32.8 and 39.9 bees/hive, respectively. On the day of drilling (but after the process was complete), mortalities averaged 40.5 bees/hive in the control field as compared to 36.5 bees/hive in the treated field. In the first 4 days after drilling, the mortality was slightly higher in the control than in the treatment group.

For the remaining assessment days, mean daily mortality of both the control and treatment groups demonstrated the same tendency to fluctuate and also demonstrated comparable timings of increases and decreases (Figure 1). Mortality peaks usually occurred simultaneously in both the control and treated plots and were usually higher in the control plots. Increases in the number of dead bees in front of the hives were mainly observed after the brood assessments that were performed during exposure in both treatment groups.

The mean daily mortality during guttation (May 28-June 26, 2009; days 6-35 after drilling) was 42.6 and 38.4 dead bees/hive in the control and treated groups, respectively.

The mean daily mortality (linen sheets + bee traps) for the entire exposure (45 days) was 39.6 and 35.7 bees/hive in the control and treated groups, respectively.

Figure 1. *Mean number of dead worker bees, pupae, and larvae/hive/day collected in the dead bee traps and on the linen sheet in front of the hives in the control and treatment groups before drilling and during the time of exposure at the test site.*



Colony condition and brood development:
At the first brood assessment, colony strength (=mean number of bees/hive) in the control hives ranged from 7,329 to 12,236 bees. Colony strength in the treatment hives ranged from 5,985 to 12,502 bees. Only the bees that were present in the hives at the time of the assessment were included in the estimates. A portion of the worker bees was outside foraging, so the estimates underestimate actual colony strength.

Colony strength in both the control and treatment group were similar during the first brood assessment, and was followed by a decrease in colony strength in the treatment group. In subsequent assessments, colony strength was similar between the control and treatment groups (Figure 2). Starting at the 6th brood assessment, colony strength decreased in both the control and treatment groups, and was more pronounced in the treatment plot.

In the control, two colonies (C1 and C4) had lower number of bees in the hives on almost all assessment dates during the entire study period. At almost every assessment, lower numbers of bees were observed in the treatment group, which is possibly explained by the time of assessment. The control colonies were always assessed earlier in the morning than the treatment

colonies when lower temperatures were present. Flight activity is reduced in low temperatures; therefore, a higher number of bees were found in the control hives. The study author reported that there was no obvious evidence of a treatment-related effect.

The development of the mean abundance of brood on the combs (eggs, larvae, and pupae) in the control was slightly higher from June 11 until the last assessment beginning in August (Figures 3 and 4). However, brood development followed the same trends in both the control and treatment group, and the values were reportedly within the range of natural variation.

The comparatively lower abundance of brood in the treatment hives in June and the beginning of July was reportedly caused by hives T2, T4, and T5. In hive T2, no queen was observed as present during two brood assessments (July 2 during exposure and July 9 after relocation), which might explain the low brood abundance.

The study author concluded that there was no obvious evidence of a treatment-related effect on brood abundance.

Only slight differences in the amount of food in the combs were observed between the control and treatment groups during the entire observation period.

Figure 2. *Mean number of honeybees per hive (=colony strength) in the control and treatment group.*

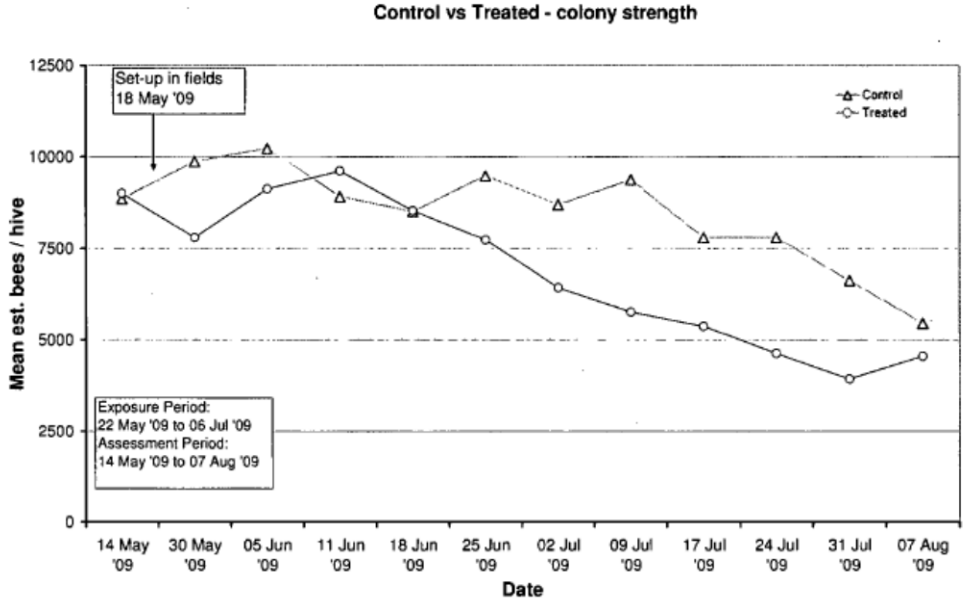


Figure 3. *Mean comb area per hive (%) covered with brood cells (eggs, larvae, and pupae) and with food stores (nectar and pollen) in the treatment group.*

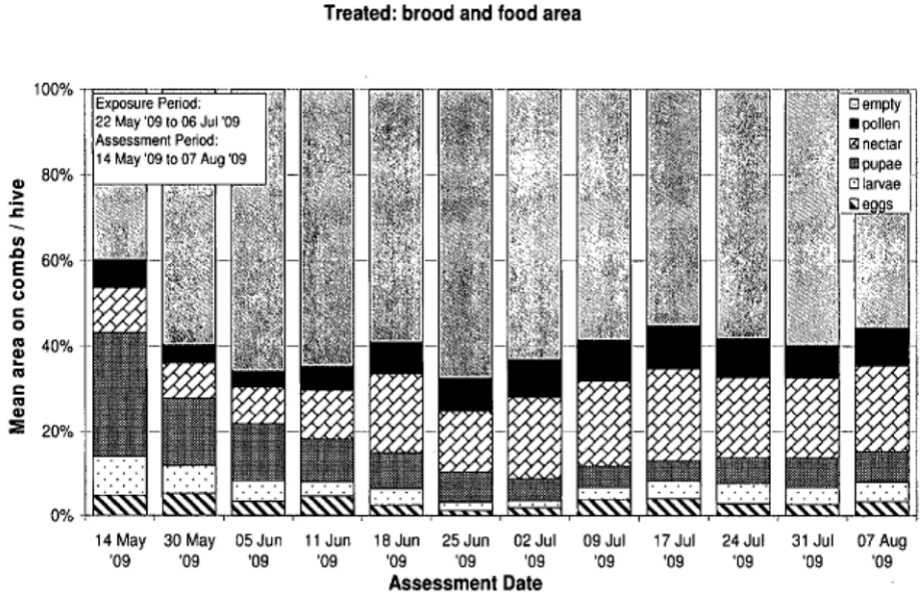
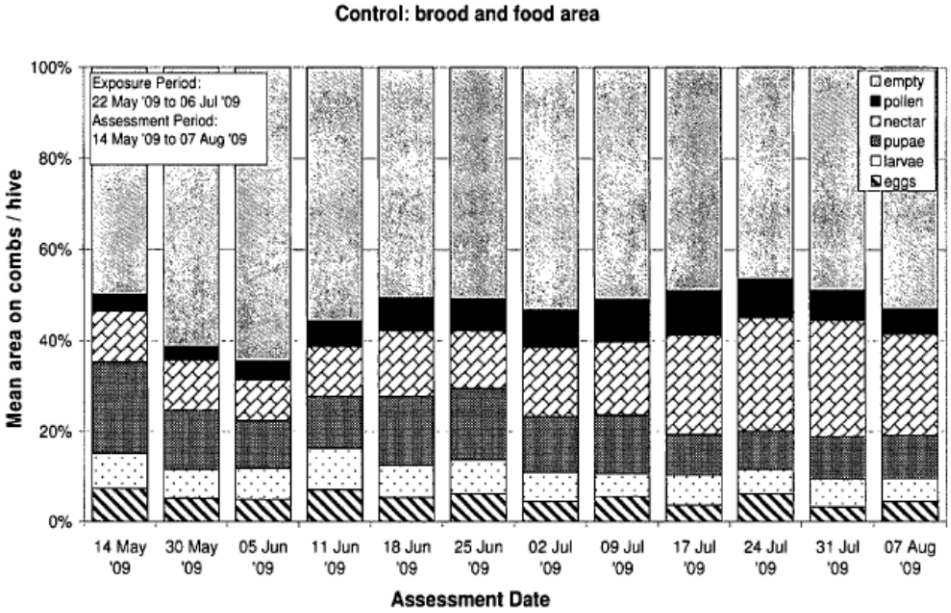


Figure 4. *Mean comb area per hive (%) covered with brood cells (eggs, larvae, and pupae) and with food stores (nectar and pollen) in the control group.*



Reported Statistical Results:

The study author did not perform statistical analysis on any of the parameters measured.

14. REVIEWER'S VERIFICATION OF STATISTICAL RESULTS:

Replicate data were provided for the bee trap mortality data when considering each individual hive as a replicate. However, individual hive data were not provided for the mortality data obtained from linen sheets placed in front of each hive. Pre-exposure mortalities (linen sheets + bee traps) were very similar between the control and treatment groups. For the exposure data, there were 3 assessment days where the treatment mortality was possibly biologically significantly higher than in the control group. However, levels of mortality were very low in both the control and treatment group; there was likely no effect of the test material.
Overall, the mean mortalities during guttation and the mean mortalities for the entire duration of exposure were very similar between the control and treatment groups; however, the treatment groups had slightly lower mean mortalities.

The reviewer visually verified the reported results and agrees with the study author's assessments with regard to colony strength due to the issues with the differing times that assessments were performed in the control and treatment groups (the control group was assessed in the morning when bee flight activity was reduced and thus more worker bees were present). The colony strength in the treatment group was comparable to that of the control group during pre-exposure and for many exposure assessments. There were 4 assessments where the control group had greater colony strengths as compared to the treatment group that might have been biologically significant. However, the timing of the assessment likely affected the available number of bees for counting at the hives as the treatment group was measured during a time of higher bee flight activity as compared to the control. Therefore, any reductions cannot be conclusively attributed to the drilling of the treated maize seeds.

The reviewer visually assessed the brood and food area data and determined that there were likely no biologically significant reductions in the treatment group present during pre-exposure with the exception of the sum area of egg cells, which was not statistically significant (a preliminary t-test yielded p=0.139). The treatment group had noticeably higher sum areas of pollen and pupal cells as compared to the control during pre-exposure. The exposure data for the control and treatment groups were visually compared, and t-tests (two-tailed, two sample) assuming equal variance were conducted using Excel 2003. Statistically significant reductions were determined for the sum area of egg cells on 34 and 63 days after drilling; however, the treatment group was already reduced in comparison to the control group before the exposure was initiated. The reviewer could not definitively determine whether the reductions could be attributed to clothianidin-dressed maize seeds.

Summary of parameters statistically analyzed by the reviewer.

Assessment day	**Treatment**	**Sum area of larval cells**	**p-value**		
20 DAD	Control	6235	0.057		
	0.506 mg ai/seed	2268			
		Sum area of egg cells	**p-value**	**Sum area of larval cells**	**p-value**
27 DAD	Control	3663	0.139	4853	0.254
	0.506 mg ai/seed	1701		2692	
		Sum area of pupal cells			
	Control	10379	0.209		
	0.506 mg ai/seed	5788			
		Sum area of egg cells	**p-value**	**Sum area of larval cells**	**p-value**
34 DAD	Control	4234	**0.019**	5154	0.069
	0.506 mg ai/seed	780		1382	
		Sum area of pupal cells	**p-value**		
	Control	10663	0.147		
	0.506 mg ai/seed	4818			
		Sum area of egg cells	**p-value**	**Sum area of larval cells**	**p-value**
41 DAD	Control	3061	0.198	4358	0.0760
	0.506 mg ai/seed	1276		1134	
		Sum area of pupal cells	**p-value**		
	Control	8431	0.126		
	0.506 mg ai/seed	3471			
		Sum area of pupal cells	**p-value**		
48 DAD	Control	8856	0.179		
	0.506 mg ai/seed	3436			
		Sum area of larval cells	**p-value**	**Sum area of pupal cells**	**p-value**
56 DAD	Control	4534	0.252	6128	0.358
	0.506 mg ai/seed	2834		3259	
		Sum area of egg cells	**p-value**		
63 DAD	Control	4215	**0.0184**		
	0.506 mg ai/seed	1949			
70 DAD		**Sum area of larval cells**	**p-value**		
	Control	4109	0.377		
	0.506 mg ai/seed	2728			

DAD= days after drilling

16. REVIEWER'S COMMENTS:

The reviewer's conclusions generally agreed with the study author's; however, the reviewer determined statistically significant reductions in the sum area of egg cells at 34 and 63 DAD for the treatment plot. There was a lower sum area of egg cells in the treated plot as compared to the control at pre-exposure. Additionally, while there were additional likely biologically significant differences present between the control and treatment groups with regard to brood measurements from May 30 to July 31, the possible issues that led to the missing queen without reported swarming activity indicate that the lower brood sum areas might have been attributable to issues with hive T2. There is no evidence that would allow the determination of whether natural causes or the clothianidin-dressed seed caused the issues with the missing queen in hive T2 or the statistically significant reductions observed for the sum area of egg cells. There were isolated incidences where differences between treatment and control hive data were found on various dates, but essentially no biologically significant differences in colony strength, mortality, and food area occurred throughout the study. There was high variability present in this study that precluded the ability of the t-tests to indicate statistical significance. As a result, there are limitations on the both the results and the reviewer's ability to determine if there was in fact a treatment related effect of clothianin-dressed maize seed on honeybees.

Climatic data (temperature, humidity, rainfall, and cloud formation) were recorded at the control field plot. Temperature and humidity during the exposure and monitoring phases were recorded taken from an official weather station in Nimes-Courbessac. Precipitation during the exposure phase was measured with rain gauges directly at the plots. During the monitoring phase, precipitation data were also obtained from the weather station in Nimes-Courbessac.

Soil samples were collected from the test fields for determination of physico-chemical properties. Five soil cores (5 cm width) were collected to a depth of 20 cm from each corner of the treated and control field plot (4 x 5 samples per field). Standard soil parameters were determined:

	Control	Treatment
Soil Type[6]	Silty loamy sand	Silty loamy sand
pH value ($CaCl_2$) [1]	7.2	7.4
WHC_{max} [g /100 g soil dry weight] [2]	45.2	48.4
CEC [mval Ba/ 100 g soil dry weight] [4]	9.2	13.0
TOC [%] [3]	1.35	1.47
Clay [%] (< 0.002 mm) [5]	9.0	11.2
Silt [%] (0.063 mm to ≥ 0.002 mm) [5]	44.2	48.9
Sand [%] (2 mm to ≥ 0.063 mm) [5]	46.7	40.0

WHC_{max} = Maximum Water Holding Capacity
CEC = Cation Exchange Capacity
TOC = Total Organic Carbon
[1]DIN ISO 10390 mod.
[2]Schaller 1993
[3]DIN ISO 10694
[4]Mehlich method mod.
[5]DIN 19683
[6]DIN 4220

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APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL ANALYSIS

Sum area of egg cells 8 DBD	*Control*	*0.506 mg ai/seed*
Mean	5006.166667	3312.833333
Variance	5826054.567	828629.3667
Observations	6	6
Pooled Variance	3327341.967	
Hypothesized Mean Difference	0	
df	10	
t Stat	1.607882714	
P(T<=t) one-tail	0.069470104	
t Critical one-tail	1.812461102	
P(T<=t) two-tail	0.138940209	
t Critical two-tail	2.228138842	

Sum area of larval cells 20 DAD	*Control*	*0.506 mg ai/seed*
Mean	6235	2267.666667
Variance	12517810	7803780.667
Observations	6	6
Pooled Variance	10160795.33	
Hypothesized Mean Difference	0	
df	10	
t Stat	2.155735466	
P(T<=t) one-tail	0.02825744	
t Critical one-tail	1.812461102	
P(T<=t) two-tail	0.05651488	
t Critical two-tail	2.228138842	

Sum area of egg cells 27 DAD	*Control*	*0.506 mg ai/seed*
Mean	3663	1700.5
Variance	6177729.2	2764242.7
Observations	6	6
Pooled Variance	4470986	
Hypothesized Mean Difference	0	
df	10	
t Stat	1.6075654	
P(T<=t) one-tail	0.069505	
t Critical one-tail	1.8124611	
P(T<=t) two-tail	0.1390099	
t Critical two-tail	2.2281388	

Sum area of larval cells 27 DAD	*Control*	*0.506 mg ai/seed*
Mean	4853.1667	2692.333333
Variance	12891537	6246097.867
Observations	6	6
Pooled Variance	9568817.2	
Hypothesized Mean Difference	0	
df	10	
t Stat	1.2099092	
P(T<=t) one-tail	0.1270689	
t Critical one-tail	1.8124611	
P(T<=t) two-tail	0.2541377	
t Critical two-tail	2.2281388	

Sum area of pupal cells 27 DAD	*Control*	*0.506 mg ai/seed*
Mean	10378.5	5788.166667
Variance	47832600	22352214.97
Observations	6	6
Pooled Variance	35092408	
Hypothesized Mean Difference	0	
df	10	
t Stat	1.3421414	
P(T<=t) one-tail	0.1046132	
t Critical one-tail	1.8124611	
P(T<=t) two-tail	0.2092264	
t Critical two-tail	2.2281388	

Sum area of egg cells 34 DAD	*control*	*0.506 mg ai/seed*
Mean	4233.6667	779.5
Variance	8042802.7	1077955.9
Observations	6	6
Pooled Variance	4560379.3	
Hypothesized Mean Difference	0	
df	10	
t Stat	2.8015826	
P(T<=t) one-tail	0.0093718	
t Critical one-tail	1.8124611	
P(T<=t) two-tail	0.0187436	
t Critical two-tail	2.2281388	

Sum area of larval cells 34 DAD	*Control*	*0.506 mg ai/seed*
Mean	5154.3333	1382
Variance	17313143	3211566
Observations	6	6
Pooled Variance	10262355	
Hypothesized Mean Difference	0	
df	10	
t Stat	2.0396102	
P(T<=t) one-tail	0.0343461	
t Critical one-tail	1.8124611	
P(T<=t) two-tail	0.0686921	
t Critical two-tail	2.2281388	

Sum area of pupal cells 34 DAD	*Control*	*0.506 mg ai/seed*
Mean	10663	4817.666667
Variance	57507302	25369582.67
Observations	6	6
Pooled Variance	41438442	
Hypothesized Mean Difference	0	
df	10	
t Stat	1.5727808	
P(T<=t) one-tail	0.0734225	
t Critical one-tail	1.8124611	
P(T<=t) two-tail	0.1468451	
t Critical two-tail	2.2281388	

Sum area of pupal cells 41 DAD	*Control*	*0.506 mg ai/seed*
Mean	8431.167	3471.333333
Variance	32793740	20133157.07
Observations	6	6
Pooled Variance	26463448	
Hypothesized Mean Difference	0	
df	10	
t Stat	1.669954	
P(T<=t) one-tail	0.062942	
t Critical one-tail	1.812461	
P(T<=t) two-tail	0.125884	
t Critical two-tail	2.228139	

Sum area of pupal cells 48 DAD	*Control*	*0.506 mg ai/seed*
Mean	8855.6667	3435.833333
Variance	45280662	39179104.17
Observations	6	6
Pooled Variance	42229883	
Hypothesized Mean Difference	0	
df	10	
t Stat	1.4445634	
P(T<=t) one-tail	0.089587	
t Critical one-tail	1.8124611	
P(T<=t) two-tail	0.1791741	
t Critical two-tail	2.2281388	

Sum area of larval cells 56 DAD	*Control*	*0.506 mg ai/seed*
Mean	4534	2834.166667
Variance	7942641.6	3806300.567
Observations	6	6
Pooled Variance	5874471.1	
Hypothesized Mean Difference	0	
df	10	
t Stat	1.2147379	
P(T<=t) one-tail	0.1261851	
t Critical one-tail	1.8124611	
P(T<=t) two-tail	0.2523701	
t Critical two-tail	2.2281388	

Sum area of pupal cells 56 DAD	*Control*	*0.506 mg ai/seed*
Mean	6128.1667	3259
Variance	28462432	24689724
Observations	6	6
Pooled Variance	26576078	
Hypothesized Mean Difference	0	
df	10	
t Stat	0.9639865	
P(T<=t) one-tail	0.1788924	
t Critical one-tail	1.8124611	
P(T<=t) two-tail	0.3577848	
t Critical two-tail	2.2281388	

Sum area of egg cells 63 DAD	*Control*	*0.506 mg ai/seed*
Mean	4215.3333	1948.5
Variance	3677891.1	223887.1
Observations	6	6
Pooled Variance	1950889.1	
Hypothesized Mean Difference	0	
df	10	
t Stat	2.81102	
P(T<=t) one-tail	0.0092213	
t Critical one-tail	1.8124611	
P(T<=t) two-tail	0.0184426	
t Critical two-tail	2.2281388	

Sum area of larval cells 70 DAD	*Control*	*0.506 mg ai/seed*
Mean	4109	2727.833333
Variance	8283703.6	5136576.167
Observations	6	6
Pooled Variance	6710139.883	
Hypothesized Mean Difference	0	
df	10	
t Stat	0.923508568	
P(T<=t) one-tail	0.188743672	
t Critical one-tail	1.812461102	
P(T<=t) two-tail	0.377487345	
t Critical two-tail	2.228138842	